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STUDIES ON THE BIOSYNTHESIS OF PENTALENOLACTONE. PART I.

APPLICATION OF LONG RANGE SELECTIVE PROTON DECOUPLING (LSPD) AND SELECTIVE ^{13}C-{^{1}H} NOE IN THE STRUCTURAL ELUCIDATION OF PENTALENOLACTONE G

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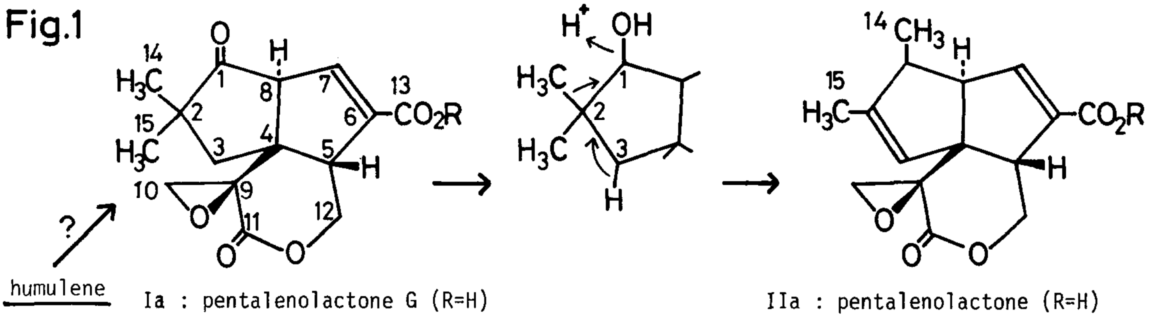
We have previously shown that long range selective proton decoupling (LSPD)[1] with a decoupler keeping at a weak power level is very useful for the assignments of ^{13}C-nmr spectra, especially of quaternary carbons. Two applications of this technique have been recently reported[2]. During these studies, one of us (J.U.) observed a phenomenon that the signal intensities of some carbons in ^{13}C-nmr spectra were enhanced upon selective irradiation of protons spacially close to the said carbons. An exhaustive study on this phenomenon using pertinent model compounds has established a new promising technique in ^{13}C-nmr spectroscopy, *viz.* selective ^{13}C-{^{1}H} NOE[3]. We wish to report herein the first application of these two techniques in the structural elucidation of a natural product.

During the course of studies on the biosynthesis of pentalenolactone, IIa, an antibiotic produced by *Streptomyces* sp.[4], we have isolated a pentalenolactone related metabolite named pentalenolactone G, Ia (G means gem-dimethyl), the structural feature of which implies strongly the sesquiterpenoidal origin of IIa (Fig. 1).

Fig.1



Ia : pentalenolactone G (R=H)
Ib : R=CH_3

IIa : pentalenolactone (R=H)
IIb : R=CH_3

After treatment with diazomethane of the $CHCl_3$ extract of the acidified fermentation broth of *Streptomyces* sp., followed by purification by preparative tlc (silica gel, benzene/ethyl acetate = 3:1, Rf value 0.38. cf. pentalenolactone methyl ester, IIb 0.61) was isolated pentalenolactone G as its methyl ester, Ib, $C_{16}H_{18}O_6$ (M^+ m/e found 306.1129, calcd. 306.1103), m.p. 125.5°, $\nu_{max}^{CDCl_3}$ 1770cm^{-1}(lactone), 1740(ketone), 1720(ester) and 1385(gem-dimethyl), no absorption between 3600-3000cm^{-1}. λ_{max}^{MeOH} 238nm(ε 6900).

The proton noise-decoupled, off-resonance and selective proton decoupled ^{13}C-nmr spectra as well as the ^{1}H-nmr spectrum of Ib[5] and comparison of these data with those of IIb, revealed the following partial structures (values show δ_H, those in parentheses are coupling constants in Hz, and those in brackets represent δ_C);

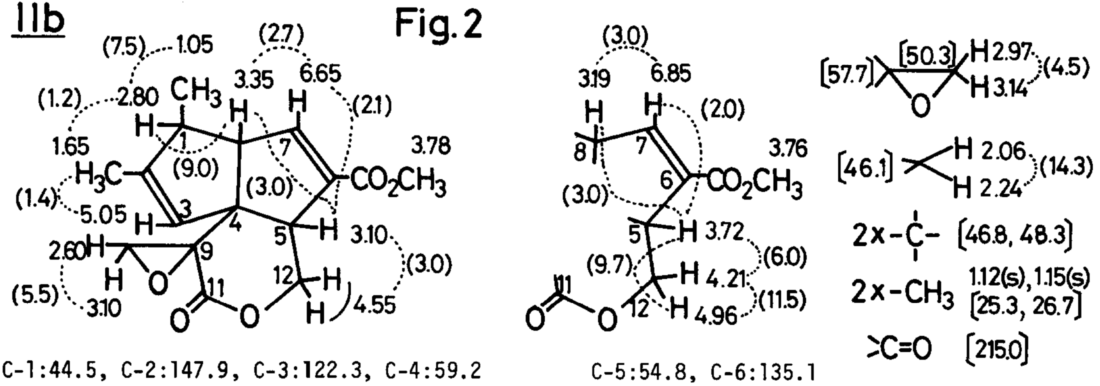


Fig.2

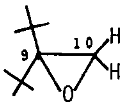
C-1:44.5, C-2:147.9, C-3:122.3, C-4:59.2
C-5:51.0, C-6:133.4, C-7:146.1, C-8:56.7
C-9:59.1, C-10:47.1, C-11:169.4, C-12:67.7
C-13:164.3, C-14:15.5, C-15:14.6, C-13':51.8

C-5:54.8, C-6:135.1
C-7:141.6, C-8:59.2
C-11:168.7, C-12:67.6
C-13':52.0

The very large coupling constant ($^1J_{C-H}$=180Hz) characteristic for three membered rings[6] revealed the presence of an epoxide, the $^1J_{C-H}$ of the corresponding epoxide in IIb being 178Hz[1]. In the above partial structures, the oxymethylene carbon (δ_C 67.6) must be connected to an ester group, since the absence of free hydroxy groups and of other oxygenated carbons but for the epoxy carbons were shown by the IR and ^{13}C-nmr spectra.

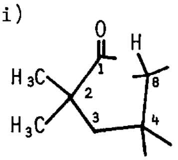
These partial structures were further extended as shown below by LSPD[1] and selective ^{13}C-{^{1}H} NOE[3] experiments and by taking coupling patterns in the ^{1}H-nmr spectrum into consideration.

(i)



In the gated decoupled spectrum (Fig. 3B), this methylene carbon (C-10) appeared as a sharp triplet and no long range coupling ($^2J_{C-H}$ or $^3J_{C-H}$) was observed. Therefore, α and β carbons of this methylene must be non-protonated ones.

(ii)



LSPD of two methyl signals (Fig. 3D) collapsed the C-3 methylene signal to a sharp triplet of doublets ($^1J_{C-H}$=125.7Hz, $^3J_{C-H}$=5.9Hz) and the C-2 quaternary carbon to a sharp triplet ($^2J_{C-H}$=3.7Hz). Since these two methyls and C-3 methylene hydrogens were not coupled each other in the ^{1}H-nmr spectrum, and since no quaternary carbons except for C-2 were decoupled, C-3 and C-2 must be three bonds and two bonds away, respectively, from the methyl hydrogens being irradiated.

In addition to the above changes, the splitting patterns of the methyl signals were simplified (Fig. 3D). This means that the protons of one methyl group are three bonds away from the other methyl carbon, i.e. the two methyl groups under consideraion are in a geminal relationship. The chemical shifts of these two carbons (δ_C 25.3 and 26.7) corroborate this conclusion[7]. Thus, the relationship of gem-dimethyl, C-2 and C-3 have been clarified.

This sequence was further extended to (ii) by the aid of selective ^{13}C-{^{1}H} NOE experiments. As shown in Table, the area of the ketone (C-1) was increased by approximately 50 % on selective proton irradiation of $C_3\underline{H_2}$ or gem-dimethyl (C-14 and C-15). Therefore, the ketone must be close to and in similar distances to both the C_3H_2 and gem-dimethyl groups, namely at the next position to C-2[8]. Irradiation of $C_3\underline{H_2}$ increased the area of a quaternary carbon resonance (δ_C 48.3) in

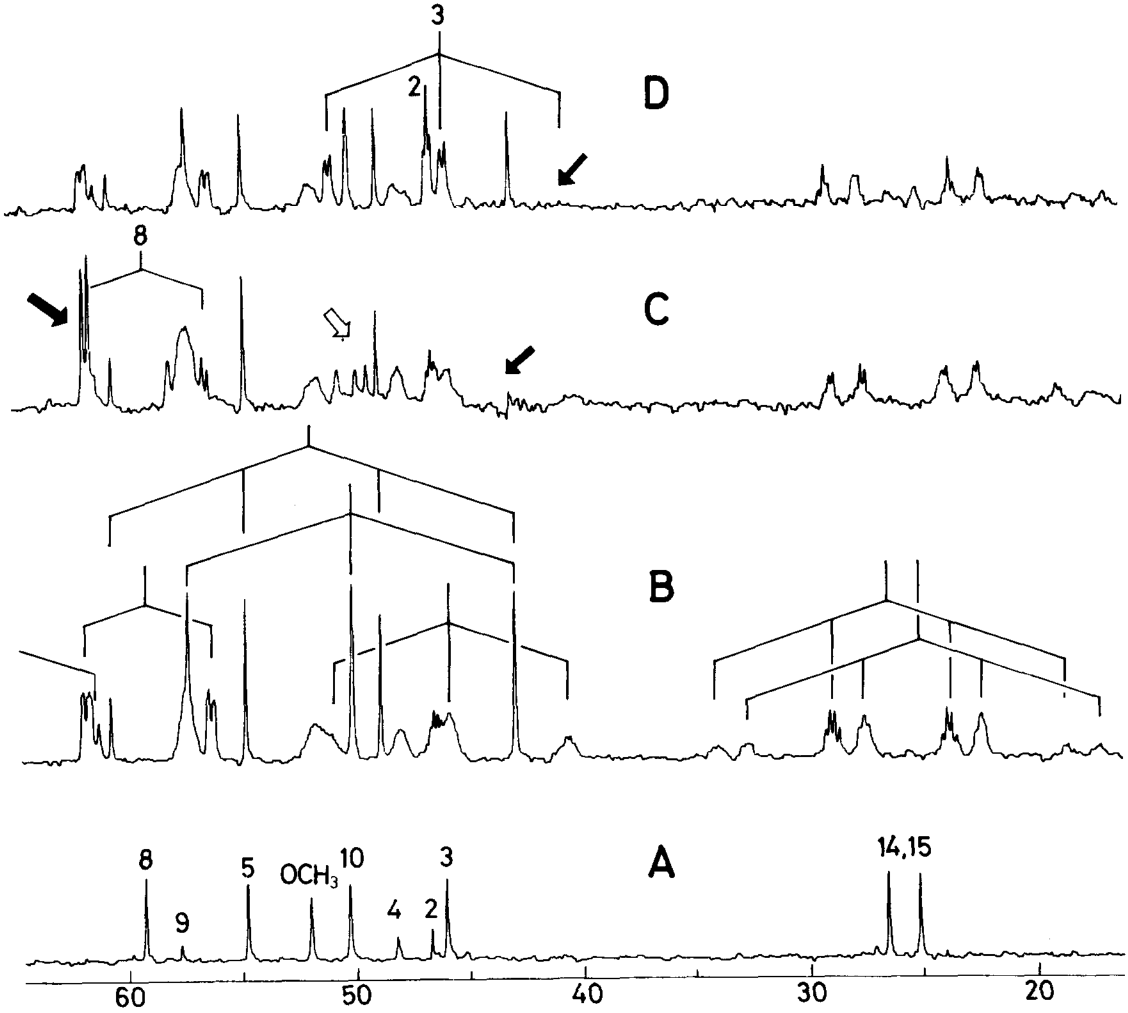


Fig. 3 Pertinent region of the ^{13}C-nmr spectra of Ib. (A) proton noise-decoupled, (B) gated decoupled, (C) LSPD at δ_H 2.15 (C_3H_2), and (D) LSPD at 1.10 (gem-dimethyl). The sample (80mg) was dissolved in $CDCl_3$ and degassed. The conditions for (C) and (D) were as follows, flip angle 45°, repetition time 2.7sec, 15000 accumulations, data points 16K, decoupler power 8 mG.

The drastic changes in the signal intensities (shown by ➡) were caused by selective population transfer[9]. A double quantum transition mechanism may be the reason of the very complicated splitting pattern in Fig. 3C (shown by ⇨)[10].

addition to that of C-2, but not those of two ester carbonyl carbons (C-11 and C-13). Therefore, C-4 could not be a carbonyl. LSPD of the same methylene eliminated the long range coupling ($^3J_{C-H}$) from C-8 (Fig. 3C). Since the protons at C-3 and C-8 were not coupled each other in the ^{1}H-nmr spectrum, these two carbons must be in a 1,3-relationship. This structural information about C-8, which has already appeared in the main fragment of the partial structures (Fig. 2), Table

^{13}C-{^1H} NOE values of Ib

	irradiated at δ_H				
bserved	1.12	2.22	4.22	4.92	6.82
carbon	CH_3	C_3H_2	$C_{12}H$	$C_{12}H$	C_7H
C-1	1.53	1.48	1.08	1.15	1.13
C-4	1.18	1.71	1.46	1.00	1.00
C-6	0.91	1.03	1.66	1.22	1.50
C-11	0.97	1.13	0.94	1.13	0.90
C-13	1.03	1.00	1.30	1.27	1.13

flip angle 15°, repetition time 2.6sec, 20000 accumulations. The ares obtained by integration were normalized to the solvent peak ($CDCl_3$).

connected most of carbon atoms in Ib. The direc combination of C-1 and C-8 was proved by the aid of chemical manipulation. Reduction of Ib with $NaBH_4$ followed by purification by tlc (benzene/ ethyl acetate = 3:1) gave a dihydro derivative which resisted crystallization ($C_{16}H_{20}O_6$, M^+ m/e 308, $M^+- H_2O$ found 290.1141, calcd. 290.1154,11) $\nu_{max}^{CDCl_3}$ 3680cm^{-1}, 1765 and 1710). In its ^{1}H-nmr spectrum, a new proton appeared at 3.75, which was coupled to C_8H (J=7.3Hz) and a hydroxy proto at 1.18 (exchangeable with D_2O).

Since the remaining bonds of the quaternary epoxy carbon (C-9) must be connected to non-protonated carbons (*vide supra*), Ib in Fig. 1 is the only possible structure for pentalenolactone G methyl ester. The structural similarity between Ib and IIb is in favour of Ib to be represented as shown in Fig. 1 including absolute stereochemistry.

Biosynthetically, IIa may be formed from Ia *via* a dihydro derivative through the mechanism as illustrated in Fig. 1. Evidences in our hand suggest that Ia may be formed from this hypothetical intermediate as a shunt pathway product.

References and Footnotes

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